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"Enhancement of Anti-Telomerase Immunity against Prostate Cancer"

W81XWH-05-1-0011 (PI Johannes Vieweg, M.D.)

Final Report

INTRODUCTION

The objectives of this award include a series of scientific studies to a) reverse tumor-mediated immunosuppression in metastatic prostate cancer patients and b) stimulate therapeutic antitumor immunity in the tumor bearing host using active immunotherapy interventions. Over the course of the grant, Dr. Vieweg and his team have explored several strategies to phenotypically and functionally define both regulatory T cells (T_{reg}) as well as myeloid-derived suppressor cells (MDSC) in the peripheral blood of advanced cancer patients. We also have developed and explored targeted pharmacological interventions to abrogate either T_{reg} or MDSC-induced immunosuppression. As shown below, considerable progress has been made in the laboratory to define these regulatory pathways and to explore these new concepts in ongoing or planned clinical studies, as described below and as demonstrated by the published data shown in references 1-4.

BODY

Treg elimination through CD25 targeting agents

In 2005, a clinical trial was performed at Duke University Medical Center (IRB # 5278-05-10R2) enrolling a total of 15 patients with metastatic prostate adenocarcinoma, stage (T₁₋₄, N+, M₀) or (T₁₋₄, N₀₋₁, M+). Due to the PI's relocation to the University of Florida (UF), the trial was prematurely closed in June 2006. Six subjects, enrolled on dose Schedule A, treatment arm A, received a single intravenous dose of denileukin diftitox (18 μ g/kg) four days prior to vaccination with LAMP hTERT mRNA-transfected DC, while a second cohort (Treatment arm B) of 6 subjects was treated with the vaccine alone. Although the targeted goal of enrollment into this trial was 24 patients, we collected sufficient data to support the primary endpoint, namely vaccine safety, of this regimen. At UF, we started analyzing T_{reg} frequencies in the peripheral blood of study patients collected prior to and after denileukin diftitox administration.

The T_{reg} depletion data and the immunological analysis from the patients that could be analyzed were detailed and provided in a prior progress report (2007-2008). These data demonstrated successful stimulation of hTERT-specific T cells in the peripheral blood of the 6 patients treated with denileukin diftitox and the hTERT vaccine.

Reversal of myeloid cell-mediated immunosuppression

Aside from T_{reg} , we have recently focused on exploring the phenotype and function of a second cellular subset present in advanced cancer patients, termed myeloid derived suppressor cells (MDSC). More specifically, we and others have shown that the growth of cancers is associated with a considerable decline in immune function. Therefore, therapeutic vaccines alone are often ineffective in overcoming tumor-mediated immune

suppression. A few prior studies have highlighted the role of MDSC in cancer-associated immune non-responsiveness in patients with metastatic RCC. Accumulation of these cells in tumor hosts is promoted by tumor-derived factors and this tumor-driven expansion of MDSC contributes to tumor escape from the immune system. In a recent manuscript [2], we analyzed the mechanisms by which MDSC inhibit T-cell responses and demonstrated reversal of immunosuppressive action by ATRA treatment *in vitro* and *in vivo*. Our results suggest that exposure to the differentiation agent ATRA facilitates depletion of MSC and improves antitumor immunity *in vivo*. These studies formed the basis for administering RCC patients with ATRA (Vesinoid) to determine dose dependent depletion of MDSC [1]. These published studies demonstrate that MDSC can be characterized by their profound immunosuppressive function and that MDSC-mediated immunosuppression can be abrogated in a dose-dependent fashion through the differentiation agent ATRA.

Oxidative stress regulates expression of vascular endothelial growth factor-1 (VEGFR1) in MDSC.

As an extension of these studies we could demonstrate that implantation of human RCC tumors into athymic nude mice promotes the appearance of VEGFR1/CD11b double-positive myeloid cells in the peripheral blood compartment. Conversely, A vastin-mediated VEGF neutralization was capable of significantly reducing the numbers of circulating VEGFR1 positive MDSC, suggesting a novel approach to enhance antitumor immunity in cancer patients. Interestingly, upregulation of VEGFR1 by myeloid cells could also be achieved *in vitro* by co-culturing bone marrow precursors with RCC-conditioned medium or by short-term exposure of naïve myeloid cells to oxidative stress. Treatment of myeloid cells with H₂O₂, the lipid peroxidation product 4-hydroxy-2(E)-nonenal, or an inhibitor of thioredoxin reductase resulted in increased cell surface expression of VEGFR1. Furthermore, we found that MDSC acquired immunosuppressive properties and became capable of inhibiting T-cell proliferation after exposure to oxidative stress. These data suggest that tumor-induced oxidative stress may promote both VEGFR1 up-regulation and immunosuppressive function in bone marrow-derived murine myeloid cells. Translating these concepts into more clinically relevant systems, we found that VEGFR1 positive myeloid cells also can be found in the peripheral blood and tumors of RCC patients. Therefore, we conclude that restoration of immunocompetence in RCC patients by pharmacological elimination of VEGFR1 positive cells may have a significant impact on the therapeutic efficacy of cancer vaccines or other immune-based therapies. For detailed experimental results, please refer to reference [2].

Generation of antigen-presenting cells from tumor-infiltrated CD11b myeloid cells.

During the conduct of the aforementioned studies, we developed the novel idea that tumor-infiltrating CD11b myeloid cells could not only act as cellular subsets mediating immunosuppression, but also may represent a novel source for immunostimulatory antigen-presenting cells. He recently investigated the possibility of generating mature antigen-presenting cells from tumor-infiltrated CD11b myeloid cells. In a recent publication, he demonstrated that *in vitro* exposure of freshly excised mouse tumors to the DNA methyltransferase inhibitor 5-AZA-2'-deoxycytidine (AZA) not only resulted in selective elimination of tumor cells, but surprisingly, also enriched CD45+ tumor-infiltrating cells. Culture of isolated tumor-infiltrated CD11b myeloid cells using AZA and

GM-CSF promoted their differentiation into mature F4/80/CD11c/MHC class II-positive antigen-presenting cells. Vaccination of mice with ex vivo generated tumor-derived APC resulted in tumor protection in 70% of treated animals. Importantly, tumor-derived APC obviated loading with exogenous antigens, but nevertheless were capable of stimulating a potent anti-tumor response. Collectively, these results demonstrated that tumor-infiltrated CD11b myeloid cells can be enriched and differentiated in the presence of the DNA demethylating agent 5-AZA-2'-deoxycytidine into mature tumor-derived antigen-presenting cells. A manuscript detailing these experimental results recently has been submitted for publication [3].

KEY RESEARCH ACCOMPLISHMENTS

- a) Successful translation of the T_{reg} depletion concept into the clinic by executing a complex phase I study and treating study subjects with a combined T_{reg} depleitory/vaccine regimen.
- b) MSDC-mediated immunosuppression could be inhibited in a dose dependent fashion through exposure to the differentiation agent ATRA (all-trans retinoic acid).
- c) Restoration of immunocompetence in advanced cancer patients by pharmacological elimination of VEGFR1 positive cells may have a significant impact on the therapeutic efficacy of cancer vaccines or other immune-based therapies.
- d) Tumor-infiltrated CD11b myeloid cells can be enriched in vitro and differentiated in the presence of the DNA demethylating agent 5-AZA-2'-deoxycytidine into mature tumor-derived antigen-presenting cells.

REFERENCES AND REPORTABLE OUTCOMES

1. Kusmartsev S, Su Z, Heiser A, Dannull J, Eruslanov E, Kubler H, Yancey D, Dahm P, and Vieweg J. Reversal of myeloid cell-mediated immunosuppression in patients with metastatic renal cell carcinoma. Clin Cancer Res 2008; 14:8270-8.
2. Kusmartsev S, Eruslanov E, Kubler H, Tseng T, Sakai Y, Su Z, Kaliberov S, Heiser A, Rosser C, Dahm P, Siemann D, and Vieweg J. Oxidative stress regulates expression of VEGFR1 in myeloid cells: link to tumor-induced immune suppression in renal cell carcinoma. J Immunol 2008; 181:346-53.
3. Daurkin I, Eruslanov E, Vieweg J, and Kusmartsev S. Generation of antigen-presenting cells from tumor-infiltrated CD11b myeloid cells with DNA demethylating agent 5-aza-2'-deoxycytidine. Cancer Immun 2009; In press.
4. Kusmartsev S, Vieweg J. Enhancing the efficacy of cancer vaccines in urologic oncology: new directions. Nat Rev Urol. 2009 Oct;6(10):540-9.

CONCLUSIONS

The studies supported by this grant have demonstrated that immunosuppressive networks exist in cancer patients. The main cellular contributors to cancer mediated immunosuppression are regulatory T cells as well as myeloid suppressor cells. Both cellular subsets can be specifically inactivated or eliminated through specific pharmacological interventions. Our data have profound implications for active immunotherapy since tumor mediated immunosuppression represent a major hurdle to stimulate curative immune responses in the cancer patient.

APPENDICES

1. Kusmartsev S, Su Z, Heiser A, Dannull J, Eruslanov E, Kubler H, Yancey D, Dahm P, and Vieweg J. Reversal of myeloid cell-mediated immunosuppression in patients with metastatic renal cell carcinoma. *Clin Cancer Res* 2008; 14:8270-8.
2. Kusmartsev S, Eruslanov E, Kubler H, Tseng T, Sakai Y, Su Z, Kaliberov S, Heiser A, Rosser C, Dahm P, Siemann D, and Vieweg J. Oxidative stress regulates expression of VEGFR1 in myeloid cells: link to tumor-induced immune suppression in renal cell carcinoma. *J Immunol* 2008; 181:346-53.
3. Daurkin I, Eruslanov E, Vieweg J, and Kusmartsev S. Generation of antigen-presenting cells from tumor-infiltrated CD11b myeloid cells with DNA demethylating agent 5-aza-2'-deoxycytidine. *Cancer Immun* 2009; In press.
4. Kusmartsev S, Vieweg J. Enhancing the efficacy of cancer vaccines in urologic oncology: new directions. *Nat Rev Urol*. 2009 Oct;6(10):540-9.